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GENERAL DIRECTIONS

Apparatus must be returned clean and in good order. The lockers will be inspected from time to time to see that the apparatus is in good condition, and this condition will influence the marks given during the course. Apparatus not in use between laboratory periods must be cleaned and put away.

Full notes of the experiments must be written in the laboratory in connection with the actual work, and never from memory afterwards. The notebooks will be called for for inspection from time to time without previous notice, and also at the end of the course. Be concise and brief, expressing results in figures, diagrams and tables where possible. Graphic records are not to be handed in loose, but must be pasted in the notebook and accompanied by suitable explanation.

CARBOHYDRATES

1. Add as much powdered dry starch ($C_6H_{10}0_5$) as you can put on a ten-cent piece, to 50 c.c. cold water. Filter 5 c.c. of this suspension and compare its appearance with the original. Add to the filtrate one or two drops (not more) of a weak solution of iodine in iodide of potassium. If starch is present, the solution will turn blue upon addition of the iodine solution.

2. Take the starch water remaining from (1) and boil for two or three minutes. Cool, filter 5 c.c. as in (1), and test for starch with the iodine solution. If the blue reaction is present, heat the test tube. Does the blue color disappear? Allow the solution to cool. What happens?

3. Test the starch solution with Fehling's solution, as follows: take 1 c.c. copper solution (No. 1), and 1 c.c. alkaline solution (No. 2), mix, and add distilled water to make 10 c.c. Boil, then add a few drops of the starch solution. Boil again. If sugar is present there will be a red precipitate and the color of the Fehling's will change.

NOTE: Whenever this test is made, the Fehling's should be boiled before the solution to be tested is added.

4. Put some starch solution in a parchment tube and place the tube in a vessel of distilled water. Allow it to stand until the next laboratory period and then test the water for starch.

5. Add a very small quantity of dextrine $(C_6H_{10}0_5)n$ to 10 c.c. of water. Why is it cloudy? Boil. Why does it become clear? To 2 c.c. of this solution carefully add alcohol. It may be necessary to add several times its volume. Result?

6. Take 5 c.c. of the dextrine solution and add one or two drops (not more) of iodine solution. Is starch present?

7. In the same way test a solution of glucose (dextrose or grape sugar, $C_6H_{12}O_6$) with iodine solution. Result?

8. Test a solution of glucose with Fehling's solution, using the same method as in (3). Result?

9. Test a solution of cane sugar $(C_{12}H_{22}0_{11})$ with iodine; with Fehling's solution. Result?

10. Take 10 c.c. of cane sugar solution, add 1 c.c. of 10% sulphuric acid (H_2SO_4) and boil 5 minutes. Add m/10 NaOH to make up the original volume, and test with Fehling's solution. Result?

11. Put some glucose solution in a parchment tube and dialyse it as in experiment (4). Test for gluocose. Result?

FATS

1. Use olive oil, and prove that fats are soluble in ether and chloroform, but insoluble in water.

2. Add to olive oil some caustic potash (KOH) and boil. A soap is formed and glycerin is set free. Saponification.

3. Shake some rancid oil with a few drops of a solution of sodium carbonate (Na_2CO_3). The whole becomes white. This is an emulsion. Examine it under the microscope and describe its appearance.

PROTEINS

1. Add strong nitric acid (HNO_3) one drop at a time to an albumin solution. A white precipitate is thrown down, which turns yellow on boiling. After cooling, add ammonia (NH_3); the yellow precipitate becomes orange.

2. Precipitate albumin with lead acetate $((C_2H_3O_2)_2Pb)$; with mercuric chloride $(HgCl_2)$; with alcohol (C_2H_5OH).

3. Heat a small quantity of albumin solution to boiling. Any coagulation? Add one or two drops of 2% acetic acid $(C_2H_4O_2)$ and boil if necessary. Result?

4. To a solution of albumin add an excess of caustic soda (NaOH), m/1, and then a few drops of very dilute solution of copper sulphate $(CuSO_4)$, m/30. Result? This is the "Biuret Reaction."

5. Take 10 c.c. of albumin solution and add 1 c.c. of a 25% sulphuric acid solution. Boil for three to five minutes, and allow it to cool. Add

water sufficient to make up the original volume. Taking a small quantity of the solution and repeat the Biuret test as above.

6. Put some albumin solution in a parchment tube and dialyse against distilled water as in the case of starch and sugar. Test the water for albumin at the next laboratory period.

PHYSIOLOGY OF NERVE AND MUSCLE

The tissues employed should be kept on a clean glass plate and should not be allowed to change the concentration of their contents through drying. The secretion from the frog's skin is injurious and contact with it is to be avoided. The nerve should be handled in such a way as to avoid mechanical injury, or chemical action, preferably by means of a clean glass rod or a camel's hair brush, wet with physiological salt solution.

The Nerve-Muscle Preparation.

''Wrap a frog in a moist cloth, the head out. Place one blade of a stout scissors in the mouth against the angle of the jaw and the other just back of the foramen magnum. Cut off the head with a single, firm movement. Pith the spinal cord by thrusting a coarse wire down the neural canal. Cut the body across transversely just back of the forelegs. Draw off the skin from the thunk and hind legs. Remove the viscera. The large sciatic plexuses will now be exposed. Lay the frog back uppermost on a clean glass plate. Push apart the semimembranosus and triceps femoris muscles and expose the sciatic nerve. Seize the tip of the urostyle with forceps and excise it up to the last vertebra, taking care not to injure the sciatic plexuses. With the scissors split the spinal column between the points of exit of the nerves. Grasp the half from which the prepared nerve comes and lift it gently, freeing the nerve with the scissors down to the knee.''

''Cut through the tendons of Achilles below the thickening of the heel. Free the muscle up to its attachment to the femur, avoiding injury to the branch of the nerve which enters its posterior surface, and cut away the remainder of the lower leg. Clear away the muscles from the femur, taking care not to injure the sciatic nerve. Cut the femur across at about the middle of its length. In the whole operation avoid stretching or pinching the nerve.''

1. Mechanical Stimulation—

Make a nerve-muscle preparation. Fasten the bone to the L of the muscle lever so that the muscle hangs vertically. Tie a thread to the tendon and attach it to the muscle lever. Adjust the nerve holder so that the nerve can rest upon a piece of filter paper kept moist with physiological salt solution. Tap the nerve near its end with the handle of a slight scalpel;

the muscle contracts. Pinch the end of the nerve with the forceps. With sharp scissors snip off the end of the nerve which has presumably been injured by the above treatment.

2. Chemical and Osmotic Stimulation—

a. Immerse the nerve in M/8 sodium chloride. After 15 minutes replace the M/8 solution by M/1. As soon as the muscle begins to twitch wash off from the nerve the concentrated solution with distilled water. When the twitching has ceased immerse in physiological salt solution. Test the irritability by mechanical stimulation.

b. Arrange the lever to write on the smoked paper of a kymograph set for slow speed. Fill the nerve holder with M/8 sodium citrate solution, but keep the muscle moist with physiological salt solution and be careful not to get any of the citrate solution on it. Mark on the drum the position of the writing point at the moment when the nerve is immersed in the citrate, and record the time. Record also the time when the muscle begins to twitch.

c. Wipe out the nerve holder and allow the nerve to dry. If twitching begins see if you can stop it by moistening with water.

3. Electrical Stimulation—

a. Galvani's Experiment—Twist one end of a piece of copper wire around the head of an iron nail. Place the nerve of a nerve-muscle preparation across the wire and touch the muscle or nerve with the point of the nail.

b. Induced Currents—Arrange the inductorium for single shocks. Mount the nerve-muscle preparation on a muscle lever adjusted to write on the kymograph. Record each contraction with the drum stationary; after each record move the drum about 5 mm. Obtain records to show: (1) The relative efficiency of make-and-break shocks as stimuli; (2) Relation between strength of stimulus and height of contraction. (Meaning of subminimal, minimal, and maximal stimuli.)

4. Opening and Closing Contraction—

Connect a dry cell with a pair of non-polarizable electrodes, placing a simple key in the circuit.

a. Place the nerve of a n-m-p in contact with the electrodes. Note that a contraction occurs on closing and on opening the key, but not during the passage of the current.

b. Place a rheocord in the circuit. Connect electrodes with the slider and one pole of the rheocord. Find a position of the slider which gives a current just below threshold and another just above. Placing the slider in the former position it may be moved smoothly to the latter without causing a contraction.

5. The Muscle Curve—

Mount a gastrocnemius without the nerve on the muscle lever and load it with a ten-gram weight. Connect the ends of the muscle to the poles of the secondary coil of an inductorium by fine copper wires. Put an electric signal and a key in the primary circuit. Arrange a writing point on a tuning fork to record hundredths of a second. Bring the three writing points into the same vertical line, and as close together as possible. It will be most convenient to have the muscle lever above and the tuning fork and signal, below.

Set the drum going at its most rapid speed, or turn by hand, and record the curve obtained from a maximal shock. Remove the tuning fork, stop the drum, but *carefully avoid disturbance of the relative position of the other two points.* The tuning fork must be allowed to mark only during the revolution in which the curve is actually made. Turn the drum cautiously until the signal is exactly at the point where the shock was given and mark the corresponding position of the muscle lever (Simultaneous ordinates). Calculate in fractions of a second (1) the latent period, (2) the period of shortening, (3) the period of relaxation.

6. Tetanus—

With the same arrangement, but moderately slow speed of drum, give a succession of stimuli by tapping the key at first slowly, then more rapidly. The record should show incomplete tetanus passing over into complete tetanus.

7. Effect of Repeated Excitation—

Inject the arterial system of a frog with a 5% solution of fuchsin S. Make two n-m-preparations.

a. Leave one gastrocnemius at rest. Mount the other in the moist chamber with the nerve on the n. p. electrodes. Load it with ten grams. Arrange the lever to write on a very slowly moving drum. Move the secondary coil outwards until the single shocks are just below "threshold value." If a succession of shocks be given a contraction occurs. Does the number of repetitions of the stimuli bear any relation to their frequency?

b. Using maximal make shocks, record a muscle curve as in (5), then stimulate rhythmically once a second. Do you get "staircase" effect? Now increase the load to forty or fifty grams. Continue the rhythmic excitation until the contractions diminish to half their height. At this stage record a second muscle curve. How does the curve differ from the first? Quickly resume the rhythmic stimulation and continue it until the contractions cease. Now stimulate the muscle directly. Were nerve, nerve-ending, and muscle equally affected by fatigue?

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c. Compare the color of the fatigued muscle with the one reserved in the fresh state. What chemical change is indicated?

8. Effect of Load on Muscular Contraction—

Arrange a gastrocnemius without the nerve, on the muscle lever, using for electrodes fine wires in contact with the ends of the muscle. Take pains to make all clamps rigid. Hang a large scale pan directly under the muscle so that its weight is supported without leverage. Using a maximal make shock, record the height of contraction with the drum stationary and the scale pan empty. Move the drum about 5 mm.; place 20 grams in the pan and stimulate. Move the drum again; add an additional 20 grams and stimulate. Continue in this way until the muscle is just unable to lift the weight. The limit of weight which the muscle can lift is called its "absolute force."

Calculate the work done in gram-millimeters, remembering that $W = \frac{lh}{m}$, where l is the load, h the height of the kymograph record, m the magnification by the writing lever.

9. Independent Irritability of Muscle; Bernard's Experiment—

a. Pith the brain of a frog and prevent bleeding by plugging the wound with a piece of match. Expose one sciatic nerve and clear it for about an inch of its length from the other tissues. Pass a stout ligature under the nerve and tie it tightly around the leg, leaving the excluded nerve uninjured. Inject into the dorsal lymph-sac a few drops of one per cent solution of curare. After paralysis has become complete, expose both sciatic nerves clear up to the vertebral column. Take up both nerves on the electrodes and stimulate. Compare results on the two sides. Now stimulate the muscles by bringing the electrodes directly into contact with them. Inferences?

b. Put a small quantity of a curare solution in a watch glass. Prepare two nerve-muscle preparations. Put the nerve only of one and the muscle only of the other into the solution. Keep the parts outside the watch glass carefully moistened with physiological salt solution. After half an hour test the irritability of each to both direct and indirect stimulation.

10. Electromotive Phenomena of Muscle—

a. Lay two nerve-muscle preparations on a clean glass plate. Designate them A and B. Lay the nerve of A lengthwise along the muscle of B. Stimulate the nerve of B at a point as far as possible from the muscle, with a submaximal single induction shock. What happens? Stimulate with the interrupted current. Prove that the "secondary contraction" is not due to escape of current.

b. Lay the nerve of a sensitive nerve-muscle preparation along a freshly exposed, vigorously beating heart. A contraction occurs at each systole.

11. Contraction of Smooth Muscle—

a. Make two transverse cuts across a frog's stomach so as to give a ring of tissue about five millimeters wide. Suspend this from a small metallic hook (a bent pin) and, by means of another hook, attach it to a light (counterpoised) lever. Make the hooks serve as electrodes by connecting fine wires to them. It usually happens that the mechanical excitation due to the cutting throws the smooth muscle into such strong tonic contraction that it may remain for an hour or more unresponsive to further stimulation. Record the contractions obtained by stimulating with the direct current and with single induction shocks. Compare these curves with those given by striated muscle.

b. Record spontaneous contractions if they occur.

CILIARY ACTION

The mucous membrane of the mouth and oesophagus of the frog is covered with cilia which normally make their effective stroke in a direction toward the stomach. Dissect out the oesophagus and with it as much as possible of the lining of the roof of the mouth. Lay it open by a ventral longitudinal split. Pin out the preparation, mucous side up, on a small piece of board. Tilt the board so that the gastric end will be elevated by a gradient of about one to ten. Place on a ciliated surface a disk of cork about 4 mm. in diameter. Is it moved? Place on the cork a 5-gram weight. Using only the cord float, find the effect of the temperature on ciliary activity; the effect of vapor of ether.

NERVOUS SYSTEM

1. Planaria—

1. With a sharp knife divide a planarian transversely in the middle of the body. Watch the movements of the two halves. In what way do they differ? Turn both pieces over on the dorsal surface. Do they regain their normal position with equal rapidity? Bisect each piece a second time and observe carefully. Is the anterior or posterior end of each segment directed forward in locomotion? What conclusion do you draw from these experiments as to the necessity of the brain in spontaneous locomotion?

2. Cover half a glass vessel with black paper and place in it several normal planarians. In another similar vessel place the halves of several transversely bisected planarians. Leave over night in such a position that the covered part of the vessel is shaded. In what part of the vessel are the animals found in the morning? Do the decapitated animals behave differently from the normal animals? Uncover the darkened half of each vessel without disturbing the contents. What is the result? Do the decapitated worms react with the same rapidity as the normal worms?

3. Retain the segments of the decapitated worms and observe the regeneration of the various parts.

2. Earthworms—

1. *Structure of the Nervous System*—Dissect an earthworm under water and make out the main facts of the arrangement of the nervous system. Draw.

2. *Locomotion*—Study the creeping movements. Observe the alternating waves of contraction of the circular and longitudinal muscles. Note the use of the setae. How does the worm creep backwards?

3. Cut the ventral nerve cord of a worm near the middle of its length, injuring the body wall as little as possible. Note carefully the effect, if any, on the coördination in locomotion. Does the brain determine the coördination?

4. *Mechanics of Coördination. Friedlander's experiment*—Cut an earthworm into two parts, and with a needle sew them together again. How is coördination between the anterior and posterior pieces now effected?

5. *Spontaeity*—Anesthetize an earthworm by placing it for a few minutes in chloroform water. (Shake up five drops of chloroform in 40 c.c. of water.) Remove the supracœsophaegal ganglion and place the worm between sheets of moist filter paper to recover from the chloroform. Keep the worm carefully for some days and study its reactions. In the meantime observe the movements and reactions of decapitated worms.

6. *Tropic Reactions*—Make observations to show that the earthworm is stereotropic. Turn a normal worm on its dorsal surface. Explain the righting reaction. Is the earthworm heliotropic? Compare the righting action and the photosensitiveness of normal and decapitated worms.

7. *Norman's Experiment.*—Prick a normal worm with a needle and note the squirming movement. Does this express pain? While a worm is creeping or quietly extended cut it in two with a single stroke of a very sharp scalpel. Note the difference of reaction of the two parts. Bisect each of these parts again in the same way and compare the behavior of the pieces anterior and posterior to the cut.

3. The Spinal Frog—

In the following experiments it is important to avoid the disturbing effects of fatigue. Frequent periods of rest are necessary. Use these intervals in the consultation of references.

The skin of the frog should always be kept moist. When not in use the animal may be placed between the folds of a wet towel.

1. *Condition and Posture*—With as little loss of blood as possible destroy the whole brain of the frog. Note the profound shock effects. Allow half an hour for recovery. Observe position of limbs, head and eyes. Do respiratory movements occur? Does the animal attempt to right itself when placed on its back?

2. *Mechanical Stimuli*—Hang up a frog by a hook in the lower jaw. Pinch the fifth toe. Is there any relation between strength of stimulus and energy of resulting movement? Is the reflex movement confined to the part (limb) stimulated?

3. *Electrical Stimulation*—Arrange an inductorium for single induction shocks. For electrodes use fine copper wires placed around one foot, and connect these with the secondary coil. The wires should not be so tight as to interfere with the circulation in the skin; but their position and pressure should be constant to insure uniform control of strength of the exciting current. Note current strength (position of secondary) at threshold of stimulation; result of stronger currents; strength necessary to produce a crossed reflex. Make the time interval between successive trials long enough to avoid summation.

4. *Summation*—Using a current just below threshold value, give a succession of shocks at the rate of five to the second. How many are necessary to cause a reflex? How is the required number affected by change in the rate? (Can you determine whether summation effect is central, or in the peripheral endings?)

5. *Thermal Stimulation*—(Make this experiment on one foot only.) Dip the toes in warm water and determine the temperature at which the reflex occurs. Does it make any difference how much surface is exposed to the warm water (depth of immersion)? Place the foot in water at room temperature and warm it very gradually. Is a reflex obtained? (Can an exposed nerve be excited by warmth or is this an effect of peripheral endings?)

6. *Chemical Stimuli*—Have brainless frog suspended as before. Holding one leg aside by a loop of thread, let the other foot dip into a beaker of M/50 H_2SO_4. Note reaction time in seconds. Instantly wash off the acid. Make four trials with suitable intervening rest periods. Are the results fairly constant? In a similar way determine the reaction time for M/25 H_2SO_4.

7. *Spreading of the Impulses*—Using a weak Faradic current as a stimulus the reflex is confined to the limb stimulated. Apply to the hind foot stimuli of increasing strength and note the order in which the other limbs are involved in the movement. Apply the excitation to a front foot. Is the order the same? Does the excitations proceed with equal readiness in the cephalad and caudad directions?

8. *Apparent Purpose in Reflexes*—Pith the brain of a fresh frog and after shock effects have disappeared suspend by a hook.

a. Place on the skin of the back, to the right of the median line, a piece of filter paper about 5 mm. square, moistened in 3 M acetic acid. The right foot will remove it. Wash off the acid promptly.

b. Apply the acid as before, but hold the foot so that it cannot be used to remove the irritant.

c. Apply the acid to various regions of the skin. Do the reflexes change according to the region stimulated?

9. *Inhibition of Reflexes*—(*a*) Exciting with weak acid as in experiment 6, find the reaction time for the foot of a spinal frog. Dip the foot again in the acid, but simultaneously pinch, or otherwise stimulate the opposite leg. The reaction time is increased. (*b*) Expose the upper end of the spinal cord and place on it some crystals of NaCl. Is the reaction time affected?

4. Reflexes in Man—

1. *Corneal Reflex*—Touch the cornea gently with a piece of clean thread.

2. *Cilio-Spinal Reflex*—Gently pinch the skin at the nape of the neck. The pupil dilates.

3. *Light Reflex*—Cover one eye for a few seconds, then uncover and note change in size of pupil.

4. *Consensual Reflex*—Cover the right eye and note dilation of left pupil. Uncover the right eye, the left pupil contracts.

5. *Accommodation Reflex*—Watch the pupil while the observed eye is accommodated for near and for distant objects respectively. (In the ''Argyll-Robertson pupil'' the accommodation reflex remains but the light is lost.)

6. *Pharyngeal Reflex*—Touch the back wall of the pharynx with a smooth, sterile glass rod. Does warming the rod to body temperature affect the response?

7. *The Knee-Jerk*—Seat the subject on the edge of a table so that the leg hangs free. Strike the patellar ligament with the edge of the hand. Repeat several times so as to judge the relative energy of the response. Try various degrees of flexion of the knee and find what angle is most effective.

Let the subject clinch the fists tightly just as the blow is about to be struck. The effect is ''re-enforced.''

5. Dependence of Skeletal Muscle on Nervous System—

1. *Tonus* (*a*) Destroy the brain of a frog. Dissect the lower part of one gastrocnemius free, cutting away the bone below the knee, but not injuring the nerve supply of the muscle. Lay bare without injury the sciatic nerve. Dissect away the thigh muscles and cut the femur. Attach the tendon Achilles to a muscle lever; tie the femur to the right angle piece of the lever, and support the body of the frog so that the nerve will not be

stretched or injured. Add a 10-gram weight. Bring the writing point into position on the kymograph and let it write a base line. Now cut the sciatic nerve and note the position of the lever after subsidence of the excitation due to the cutting of the nerve.

(*b*) Repeat on the other leg, except that instead of cutting the sciatic you destroy the spinal cord after you have preparation mounted so that the lever will indicate any change in the length of the muscle.

2. Clamp the ergograph to the table. Attach a long straw to the spring. With the forefinger placed as near as possible to the vertical support, raise the writing point as high as possible. Note the position and set the tuning fork a little above. Record simultaneously on a rapid drum the tuning fork curve and the maximal curve of maximal muscular effect. The latter curve will contain rhythmical vibrations. Determine their average number per second. These probably represent individual discharges from the spinal nerve cells. Why?

6. Experiments on the Brain—

(Before beginning the experiments the student should dissect a frog's head carefully, noting especially the relations of the various parts to the external topography.)

1. *Removal of the Cerebral Hemispheres*—Cleanse the hands and instruments thoroughly with soap and water; then place the instruments on a clean towel in boiling water. The instruments may then be removed without touching with the hands by lifting the towel by its corners. Anesthetize a frog by placing it under a finger bowl with a small wad of absorbent cotton moistened with ether. Cleanse the skin of the dorsal surface of the head, using first soap and water, followed by *very weak* formaldehyde or mercuric chloride, wrap the frog in a wet, sterilized cotton cloth, leaving the head only uncovered.

Make a transverse incision through the skin of the head on a line with the front edges of the tympanic membranes. From this cut make a median incision forward nearly to the nostrils. With scissors or forceps carefully cut into the extreme anterior end of the cranial cavity. Insert cautiously one blade of a small, strong forceps and chip off the bone bit by bit till the hemispheres are exposed. With a very sharp instrument sever the connection between the hemispheres and the optic lobes, taking great care not to injure the thalami. Bring the skin into place and sew with fine silk thread.

The animals should be kept as many days as possible. Make notes of their condition (*a*) immediately after the operation, (*b*) after an hour or two, and (*c*) one or two days after. The frogs may be kept in pans covered with wire netting. Just enough water should be furnished to keep the bottom of the pan covered. Use great cleanliness throughout.

Compare the decerebrate frog with a normal frog in the following particulars:

(*a*) Spontaneity.
(*b*) Coördination of movements.
(*c*) Reflexes to touch on various parts of the body, especially.
(*d*) Croak reflex.
(*e*) Vision.
(*f*) Balance experiment.

7. Dermal Sensations—

A. *Touch and Pressure*—

1. Use hairs or bristles of different diameters and press lightly on various parts of the skin (finger tips, palm and back of hand, wrist, back and front of forearm, forehead, cheek, lips, tip of tongue, ear, nose, back of neck). Carefully determine which parts are most sensitive. Apply 5% carbolic acid to the finger and repeat. Why is a slight touch to the cornea painful?

2. By means of compasses determine the distances at which the points must be placed from each other in order that they may be felt as two points when applied lightly and with the same force to various parts of the body. (See list in 1.)

3. Dip finger or hand into water at the body temperature. Where is the pressure felt? Repeat this with mercury. Explain. Arrange a tuning-fork so that a bristle fastened to it strikes a touch-point intermittently. Are the vibrations felt separately? Repeat this with a vibrating string.

B. *Temperature*—

1. Using a metal point cooled in ice, determine the distribution of cold points on various parts of the skin. Make careful diagram of 1 sq. cm. of back of hand.

2. Using a hot point, determine in the same way the distribution of warmth points. Make diagram of 1 sq. cm. of back of hand. Which points (cold or warm) are mostly sharply defined? Why? Which are more numerous? Have they any relation to hairs?

3. Place a piece of wood and a piece of iron on ice until they are thoroughly and equally chilled. Which feels the colder? Why? Repeat, using heat instead of cold. Test the sensation caused by dipping the finger into water and mercury at the same temperature.

8. Hearing—

1. *Acuteness of hearing*—In a quiet room blindfolded the subject and close one ear with cotton. Hold a watch opposite the other ear and deter-

mine the distance at which the ticking can just be heard. Do the same for the other ear.

2. *Judgment of direction of sound*—(The subject sits with eyes closed.)

(*a*) Hold the watch at various positions near the head and have the subject state the location.

(*a*) Close one ear. Effect on accuracy of localization?

(*b*) Have the subject place the edges of his hands against the temples just in front of the ears, the palms directed backward. Does this affect localization?

3. *Bone conduction*—Hold a ticking watch between the teeth. Close both ears with the fingers. What change in the auditory sensations? Unstop one ear. Does the location or quantity of the sensation appear to change?

9. Taste—

1. Dry the tongue thoroughly. Place on it a small crystal of sugar and note that there is no sensation of taste till some of the substance is dissolved.

2. With a pointed camel's hair brush apply 20 per cent cane sugar solution to various points on the surface of the tongue and map out the relative sensitiveness of the various parts. Do the same with a dilute solution of quinine sulphate.

3. Repeat the test, using salines and acids.

10. Physiological Optics—

A. *Reflection and Refraction*—

1. Make diagrams showing the formation of the image by reflection from a convex and from a concave mirror.

2. Determine by construction the length of the image of an arrow 2 cm. long placed 10 cm. from the middle point of a concave mirror of 5 cm. radius of curvature.

3. Place a block of glass on a sheet of paper and mark out its outlines. Fix two pins so that the line joining them forms an acute angle with one surface of the glass. Place the eye on the opposite side of the glass so that the two pins seen seem in line with one another. Now place two other pins on the side next to you so that all four seem to be in the same straight line. Draw lines between the pins and explain by diagrams the resulting phenomena.

4. Repeat experiment 3, using a prism instead of the glass block.

5. Make diagrams to illustrate the formation of images by concave and by convex lenses, in such a way as to illustrate the meaning of the follow-

ing terms: Principal and conjugate foci, virtual image, spherical aberration, dispersion circles, chromatic aberration.

B. *Accommodation*—

1. Stick two pins in an upright position on the side of a wooden rod so that they shall be about half a meter apart. Close one eye. Hold the rod so that the pins shall be almost in line, the near pin being about 15 to 20 cm. from the open eye. Look at the near pin, the distant one becomes indistinct. Accommodate for the far pin, and the near one ceases to be distinct. Make diagrams to explain.

2. Observe carefully the pupil of another student while he accommodates for near and for far objects. Explain the advantage of the change which occurs. Observe the pupil and iris of the subject as seen from the side which he accommodates for near and distant objects. Notice that the change in position of the iris due to the thickening of the lens may be seen when the eye is adjusted for near vision. In making this observation it is important that the subject shall not change the position of the eyeball.

3. *Determination of Near and Far Points*—(*a*) Cut two corks so that they slide along a ruler. Mount a needle vertically on one cork and a card with two holes in a horizontal line on the other cork. Place the needle about 25 cm. from the card. Close one eye. Look through the two holes as in Scheiner's experiments: and when one distinct image of the needle is seen, gradually bring the needle toward the card. Observe that it becomes double at a certain distance from the eye. Why? This is the *near point of accommodation.* Make several trials and measurements. Record average in both centimeters and inches.

(*b*) Determine in similar manner the near point with a horizontal needle and card with the holes vertical. The average measurements of (*a*) and (*b*) may differ. Why?

C. *Blind Spot*—

a. Hold card No. A vertically about ten inches from the right eye, the left eye being closed. Look steadily at the cross with right eye, when both cross and circle will be seen. Gradually bring the card towards the eye, keeping the axis of vision fixed on the cross. At a certain distance the circle will disappear.

b. Make a black cross on a piece of white paper placed before you on the table. Rest the forehead on a ring on the iron stand, so arranged that the eye is exactly 30 cm. above in a vertical line with the cross on the paper. Fix the center of the cross with one eye, the other being closed, and move over the paper a pencil covered, except at the point, with white paper, until the point just disappears. Make a mark on the paper at this point and repeat the observations for all diameters of the field. The blind spot will be thus marked out. Its size and distance from the fovea centralis are to be calculated by the construction method.

D. *The Macula Lutea or Yellow Spot. Maxwell's Experiment*—

Make a strong solution of chrome alum, filter it, and place it in a clear glass bottle with flat sides. Close the eyes for a minute or so, open them, and while holding the chrome alum solution between one eye and a white cloud, look through the solution. An oval or round rose spot will be seen in the otherwise green field of vision. The pigment in the yellow spot absorbs the blue-green rays, hence the remaining rays which pass through the chorme alum solution give a rose color.

E. *Binocular Vision*—

Coördination of Accommodation and Convergence—Having both eyes open, hold a pencil a foot from them and look at a distant object. The pencil appears double. Close or cover the right eye. Which image disappears? Why? Close the left. Which image disappears? Why? Reverse the experiment by accommodating for the near object. The far object will appear double. Close one eye. Which image disappears? This experiment is easiest done by having one object 20 to 30 cm. from the eye and the other 45 to 60 cm. away. Make diagrams for these experiments.

PHYSIOLOGY OF THE BLOOD

1. Examine a drop of frog's blood under the microscope. Note the red and white corpuscles, their size, shape, and color. Is a nucleus present in both varieties?

The blood should be diluted with physiological salt solution and examined in a thin layer under a cover glass.

2. Examine in the same way a drop of mammalian blood. Describe differences.

3. Take three test tubes and mark them A, B and C. Put in tube A, 10 c.c. M/3 NaCl solution; in tube B, 10 c.c. M/6 NaCl solution; in tube C, 10 c.c. M/12 NaCl solution. Add one drop of blood to A, B, and C. Note the appearance of each tube. Why is tube C transparent? Examine a drop from each tube under the microscope and note carefully the appearance of the red corpuscles. Make drawings.

4. Take 5 c.c. "salted plasma" in a test tube and slowly add M/12 NaCl drop by drop until the blood coagulates.

5. Take 5 c.c. "oxalate plasma" in a test tube and add a little calcium chloride ($CaCl_2$). Result?

PHYSIOLOGY OF THE CIRCULATION

1. The Terrapin Heart in Situ—

Pith the brain of a terrapin. Remove the plastron. The heart will be seen through the transparent pericardium. The beating may be inhibited

through the effect of the operation; if so it will be resumed after a few minutes. With fine forceps seize the pericardium and cut it through, avoiding blood vessels visible on its surface. The *ventricle* will be seen as a large pink mass, its transverse dimension greater than its length. Note the *large arteries,* pulmonic and aortic, curving cephalad and dorsad from the base of the heart. From the apex a slender thread of connective tissue, the frenum, passes to the pericardium. Tie a fine thread on the frenum. Cut it loose from the pericardium. By means of this thread the ventricle can be gently moved about sufficiently to see the various parts. In addition to the structures mentioned, note:

(*a*) The two thin-walled *auricles,* each partially hidden by the ventricle.

(*b*) The *sinus venosus,* a V-shaped, very thin-walled structure, is seen by gently raising the ventricle. It receives the *posterior vena cava* and the two *anterior venae cavae.*

Observe: (1) Which parts contract rhythmically.

(2) The sequence of action of the parts.

(3) The rate.

(4) The proportionate time approximately of the systole of the various parts.

(5) The change of color in systole and diastole.

(6) The change of shape of the ventricle.

2. Automatism of the Heart—

a. Remove the whole heart by cutting widely around the sinus and great veins and arteries. Keep moist with Ringer's fluid. Compare the rate of beat with that in situ.

b. With *very sharp* instrument cut between sinus and right auricle. Do the separated parts beat? Rate of each.

c. Cut between the auricles and ventricle.

d. Cut off the basal fourth of the ventricle. Prick the apical part with a needle. Touch it with a pair of electrodes connected with a dry cell. Place on it a very small crystal of NaCl. Does a continuously acting stimulus cause a tetanic contraction. Effect of the interrupted current?

3. All Contractions Maximal—

Mount a fresh apical strip of the heart on the L of the muscle-lever and counterpoise. Connect the end of the strip with the poles of the inductorium by means of fine copper wires. Arrange for single induction shocks. Place the writing point against the smoked surface of the kymograph with drum stationary. Find the strength of the shock which will just cause a contrac-

tion and record the height. Wait 20 seconds; turn the drum about 5 mm. Stimulate with a stronger shock. Continue to increase the stimulus. Is the height of contraction increased?

4. Staircase—

Use the preparation already employed in experiment 3, after allowing it to rest for 5 minutes. Arrange for a stimulus a little above threshold. Repeat at intervals of 5 seconds. Explain the relation of experiments 3 and 4.

5. Refractory Period—

Without removing the beating heart attach it to a lever so as to record its contractions. Fasten a pair of fine wires to the base and apex respectively and connect with the secondary coil of the inductorium. Place a signal magnet and simple key in the primary circuit. Bring the two writing points as nearly as possible in the same vertical line.

Stimulate the ventricle with minimal single shocks. Time the closing and opening of the key so as to catch the ventricle in various stages of its cycle. Are results alike when the stimulus is given in diastole and systole?

6. The Effect of Temperature—

Remove the whole heart by cutting widely around the sinus and great veins and arteries. Use a glass rod with the "L" downward, and attach the heart by this means to a muscle lever. Observe the number of beats per minute at room temperature, then immerse the heart in Ringer's solution at a temperature 10 degrees below room temperature, and again observe the number of beats per minute. Make observations at various temperatures 10 degrees apart.

7. The Nerves of the Heart—

1. *The Vagus*—Pith the brain of a tortoise. Remove the plastron. In the neck lay bare the vagus, which will be found accompanied by the cervical sympathetic near the carotid. Pass a thread from the apex of the ventricle to a lever arranged to write on a small drum. Record the time of stimulation by signal magnet in the primary circuit.

Raise up the vagus on the platinum electrodes and stimulate with the interrupted current. Note (*a*) the latent period (in heart beats); (*b*) tonus changes; (*c*) inhibition; (*d*) after effect; (*e*) irritability during inhibition.

2. *Inhibitory Ganglion*—By stimulating at the sino-auricular junction the heart may be brought to a standstill with the same phenomena as in vagus excitation.

3. *Connection of the Inhibitory Nerves*—(*a*) Having found the strength of stimulus necessary to insure vagus inhibition, paint the heart at the region of the inhibitory ganglion with a 1% solution of nicotine. After a few minutes it will be found that the heart can be inhibited by excitation of the sino-auricular junction but not by stimulation of the vagus. Why? (*b*) Paint the heart with a 1% solution of atropine. Can inhibition now be brought about? Why?

8. Effect of Salts on Heart—

In the following experiment students should work in groups of eight, each group being subdivided into groups of two each, designated as sub-group A, B, C and D. Each student must get clearly the results of all the others.

Sub-group A—Prepare a strip of tortoise ventricle and mount it on a heart lever in such a way as to permit of its immersion. Arrange to record contractions on a *very* slow drum. When all is ready, immerse the strip in a M/8 solution of NaCl, made up with distilled water, and allow it to remain until all contractions cease. Note change in TONUS as well as individual beats. When all contractions have ceased add 0.5 c.c. H_2O_2 to the solution, and note the result.

Sub-group B—Proceed as in (A), but when the beats have begun, change to a solution containing 50 parts NaCl, plus 1 part KCl. Compare with (A) as to length of time contractions continue, tonus, etc.

Sub-group C—Proceed as in (A), but when the beats have begun, change to a solution containing 50 parts NaCl, plus 1 part $CaCl_2$. Compare with A and B.

Sub-group D—Proceed as in (A), but when the beats have begun, change to Ringer's solution (50 NaCl; 1 KCl; 1 $CaCl_2$). Compare with A, B and C.

9. Haemodynamics—

1. *Circulation in Frog's Web*—Select a small frog with little pigment. Wrap the animal around with a wet cloth, allowing one foot to protrude. Tie threads on two adjacent toes and gently spread the membrane over the glass of the web board. *Do not stretch too tightly.*

Can you distinguish arteries, veins, and capillaries? State the differences observed (*a*) in rate of flow, (*b*) in direction, (*c*) in pulsation. (Better results may be obtained by study of the flow in the mesentery of a lightly curarized frog.) Take plenty of time to make these observations carefully.

2. *The Circulation Scheme*—The bulb represents the ventricle, the valves are the mitral and semilunar respectively. The porous rattan serves as capillaries. A side branch permits flow past the capillaries. Mercury manometers indicate relative arterial and venous pressures. The large thin-walled tube represents the aorta.

a. Open the side branch by unscrewing the pressure clip. See that the tubes are well filled with water. Make a single brief pressure on the ventricle.

Note: (1) That practically all the liquid driven out by the stroke escapes through the side branch, in which the resistance is low, rather than through the high capillary resistance. (2) Only a portion of the liquid escapes during the stroke. (3) The portion which cannot escape past the resistance during the stroke finds space in a very evident dilation of the tubes nearer the pump. (4) A membrane manometer coupled to the side tube of the ventricle would show a sudden rise and fall indicating a sudden rise and fall of intraventricular pressure. (5) Close observation shows that on the stroke of the pump the tubing just distal to the pump begins to expand sooner than that farther away. (6) The arterial manometer shows a sudden rise and fall. The oscillations of mercury following the fall are due to inertia. (7) Observe the action of the valves. (8) Place a finger on the aorta and note the pressure wave pulse as it passes along the vessel.

b. With the side branch open as in experiment 1, compress the bulb rhythmically and gradually increase the frequency of the strokes. It will be found that at about twenty strokes to the minute the stream will be intermittent. As the interval between the strokes is shortened the liquid received from the pump in any one stroke cannot all escape past the resistance during the stroke and the succeeding interval.—The stream becomes remittent.

Note that the arterial pressure remains low even after the stream becomes continuous.

c. Close the side branch, so that the liquid must pass through a high peripheral resistance. Compress the bulb at such a rate that the outflow shall be continuous. The frequency required to make the flow continuous is now much less than when the peripheral resistance was low.

Allow the manometers to come to equilibrium, then compress the bulb at a rate that will produce a continuous outflow. The arterial manometer registers a higher and higher pressure, until at length the pressure ceases to rise. The pressure now suffices to drive through the resistance during each stroke and the succeeding interval all the liquid received from the pump during the stroke.

The venous pressure remains very low.

e. With the aid of the circulation scheme study the events of the cardiac cycle. Make out the following points: (*a*) Is there any time in which both valves are open? (*b*) Both closed? (*c*) Relative pressures in ventricle and aorta when semilunar valves are open? and when closed? (*d*) Relative pressure when mitral valve is open and closed?

f. Comparing the preceding experiments make a tabulated statement of the effect on blood pressure of each of the three variables, rate of heart beat, strength of contraction, and peripheral resistance.

10. The Pulse—

Place the tips of the first three fingers over the radial artery. Note: The rate. How affected by posture? By exercise?

PHYSIOLOGY OF RESPIRATION

1. The Respiration Scheme—

The rubber balloon represents the lungs. The size of the pleural cavity is regulated by the water-level within, which is controlled by the position of the funnel. The manometers represent intrapulmonary and intrapleural pressures respectively. In using the apparatus the balloon should be slightly stretched even in expiration.

1. *Normal Respiratory Movements*—Raise the water-level (diaphragm) until the balloon is only very slightly stretched. Close the pleural tube. With a glass rod reduce the glottis to a very small opening. Slowly raise and lower the diaphragm and note the relative pressures indicated by the manometers:

(*a*) During inspiration.
(*b*) In the period of equilibrium at the end of inspiration.
(*c*) During expiration.
(*d*) In equilibrium at the end of expiration.

2. Retaining the small size of glottis, study the effect of varying the rate of respiration on the magnitude of the pressure changes.

3. Enlarge the glottis. How does this affect the pressure changes?

4. *Coughing*—Remove the glass rod, but completely close the glottis with the fingers. Rapidly raise the diaphragm and while doing so, suddenly open the glottis.

5. *Hiccough*—Close the glottis with the lung in the expiratory phase and suddenly open it near the end of a strong movement of inspiration. Make a careful statement of the pressure changes during the respiratory cycle in normal respiration and also in the modified forms.

6. Open the pleural tube and repeat the respiratory movements. How are the pressures and lung movements affected?

2. Respiratory Rhythm—

By means of a pneumograph and Marey's tambour secure a graphic record of the respiratory movements. Compare the relative duration of inspiration and expiration. How is the curve modified by laughing? Coughing? Sipping water? Do not allow the subject to see the curves while in progress.

3. Exchange of Gases through an Animal Membrane—

(*a* and *b* to be done by alternating pairs of students.)

a. Dissect out the lungs of a tortoise, taking care to avoid punctures. (Test by inflating under water.) Press out the contained gases; fill with ordinary air, and suspend in a jar of CO_2 gas. See whether any change in volume occurs within an hour, but preserve till the next day.

b. Fill a similar preparation with CO_2 and suspend in ordinary air. What difference? Explain fully.

4. Relation of Heart Beat to Respiration—

Arrange to record the carotid pulse simultaneously with the pneumograph. Find the ratio of pulse to respiration. Vary the rate and depth of respiration. How is the heart beat affected?

5. The Respiratory Center—

1. Very carefully expose the medulla of a frog. Wait for the normal breathing movements to be well established. Record the rate of the breathing. Now apply carefully a few drops of M/10 HCL to the surface of the medulla. (It is important to avoid allowing the acid to touch the skin of the frog.) How is the rate affected?

6. Speck's Experiment—

Determine the number of seconds which you can hold the breath without marked discomfort.

a. After an ordinary expiration.
b. After an ordinary inspiration.
c. After a very deep expiration.
d. After a very deep inspiration.
e. After several very deep inspirations.

7. Chest and Lung Measurements—

Record in your note book the following data:

1. Your height.
2. Weight.
3. (Measured by the spirometer.)
    a. Vital capacity.
    b. Tidal air.
    c. Complemental air.
    d. Supplemental air.
4. (Measured with meter tape.)
    1. Girth of chest over nipples.
        a. At forced inspiration.
        b. At forced expiration.
        c. In repose.

PHYSIOLOGY OF DIGESTION

1. Saliva should be collected an hour or two after a meal. Rinse the mouth with water and inhale a few whiffs of the vapor of ether or acetic

acid to cause a reflex secretion of saliva. Collect in a beaker or test tube, with as few air bubbles as possible. Note its appearance and test its reaction with litmus paper.

2. Dilute some saliva with five volumes of water. Prepare three test tubes, A, B, and C. In A place some starch solution; in B place an equal volume of saliva, and in C place 3 volumes of starch solution plus 1 volume of saliva. Water bath at 40° C for ten minutes, then test each tube for starch and for sugar. Results?

3. Prepare three test tubes, A, B, and C. In A put some saliva, boil and add starch solution as in (2-C). In B place the same quantity of starch and saliva, but add to it a few drops of HCl. In C put the same quantity of starch and saliva and add a few drops of KOH. Water bath at 40° C for ten to fifteen minutes and test for sugar. Results?

4. Repeat experiment (3-C) with various concentrations of KOH, and at various temperatures, and determine the optimum alkalinity and temperature for saliva.

5. Label three test tubes as before. Put an equal quantity of artificial gastric juice in each tube. Test the reaction of each tube. Boil tube B. Cautiously add Na_2CO_3 to tube C until its reaction is alkaline to litmus paper. Add water to tubes A and B until their volume is equal to that of tube C. Add a small cube of hard-boiled egg white or fibrin to each tube. Water bath for an hour at 40° C. Note appearance of each tube and test for products of digestion. Has any digestion taken place? Set aside for next class day and repeat the tests.

6. Prepare three test tubes as before. Put an equal quantity of pancreatic extract in each tube. To tube A add some starch solution. To tubes B and C add a small cube of hard-boiled egg white or fibrin. Also add to tube B sufficient HCl to make the pancreatic extract acid in reaction. Set aside until next class day. Then test for starch and sugar in tube A and for albumin and peptone in tubes B and C.

7. *Lipase and the Reversibility of its Action*—

(*a*) Prepare about 10 grams of fresh pig's pancreas as free from fat as possible. Grind it thoroughly with sand in a mortar. Add 50 c.c. of distilled water and filter through two or three thicknesses of cheesecloth. *Always shake this solution before using.*

Label three test tubes A, B, and C. Dilute some of your lipase solution with its volume of water. In tube A place 5 c.c. of the dilute pancreas extract and *exactly* 5 drops of ethyl butyrate. Cork tightly, place the tube in a water bath at 40° C for fifteen minutes. Remove to ice water until thoroughly cold. Add a few drops of neutral litmus solution. Note the reaction. It is acid. Now titrate with M/20 NaOH, i.e., add the alkali from burette until the litmus just changes blue. Note the amount of alkali required.

To the tube B add 5 c.c. of the dilute pancreas extract that has been boiled and cooled. Also 5 drops of ethyl butyrate. Cork and place in warm bath as in the case of tube A. Leave fifteen minutes, cool in ice water and titrate in the same way as tube A. Note the amount of alkali needed to neutralize the mixture in tube and compare same with amounts used in tubes A and C.

To tube C add 5 c.c. of unboiled dilute pancreas extract. Place in ice water until cold and then titrate as described for tube A. This will determine the acidity of the pancreas extract. Compare results of titration of the three tubes and draw conclusions as to the effect of pancreas extract, boiled and unboiled, on the fat, ethyl butyrate. What is the effect of boiling on an enzyme solution?

(*b*) *Reversible Action*—Mix 25 c.c. of M/40 solution of butyric acid and 10 c.c. of 30 per cent alcohol. Divide the mixture into two equal parts and place in two flasks. To one flask add 5 c.c. of strong pancreas extract; in the other flask place the same amount of pancreas extract previously boiled and cooled. Cork both flasks tightly and place in the water bath at 40° C for fifteen minutes or more. Compare the odor from the two flasks. Does either give the odor of ethyl butyrate? How could that substance be formed?

8. ''*Litmus Milk*'' *Test*—Put 10 c.c. of milk in each of two test tubes and add a small amount of litmus powder. To one tube add also 3 c.c. of neutral pancreatic extract, and to the other 3 c.c. of water, or boiled pancreatic extract. Water bath at 40° C and note any changes. Explain results.

9. *The Clotting of Milk by Rennet*—Warm 25 c.c. of milk to about 35° and add 1 c.c. of rennet solution; stand for awhile at 35° C.

Put 25 c.c. of ''oxalated milk'' in each of two test tubes. To one add 1 c.c. of the rennet solution and to the other 2 c.c. of 2 per cent calcium chloride solution and 1 c.c. of the rennet solution. Set tubes aside at 35° C.

Boil a little of the rennet solution and add 1 c.c. of this to 10 c.c. of milk and set aside at 35° C.

Examine and compare the tubes from time to time.

URINE ANALYSIS

1. Take 25 c.c. of urine and cautiously add a few drops of nitrate of silver ($AgNO_3$). A precipitate of silver chloride will be formed, showing the presence of chlorides. Filter this solution and add to the filtrate ammonium hydroxide (NH_4OH). The precipitate shows the presence of phosphates. Again filter and add to the filtrate a small quantity of barium chloride ($BaCl_2$). To precipitate will indicate the presence of sulphates.

2. To a sample of urine add some sodium hypobromite. Bubbles of gas (nitrogen) will be given off, showing the presence of nitrogenous bodies.

3. Test a sample of urine for sugar; for albumin.

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